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ESTIMATION, RECOVERY STUDY & ASSAY OF MARKETED DOSAGE FORM OF BUPROPION HYDROCHLORIDE THROUGH DERIVATIVE SPECTROSCOPIC METHOD

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ABSTRACT: Depression is the most common and spreadable disorder in recent age as people are going through a lot of pressure in their daily work caliber and so many unconditional events. A new approach called derivatization spectroscopy was developed for the anti-depressant agent, marketed formulation of Bupropion hydrochloride, to confirm the interfering substances from the excipients of solid dosage form, in addition to that the stability parameters to assure the analysis time with the minimum usage of the reference standard and sample solution. The present work aims to develop simple, sensitive stability indicating the method to develop a model to estimate BPH in the solid dosage form by using derivative spectroscopy tool to avoid the excipients interferences in the developed assay method. First, made the stock solution of 100 µg/ml from 1000 µg/ml previously prepared solution. The method was validated over the concentration range of 5-50 µg/ml of marketed formulation of Bupropion Hydrochloride. The assay method & derivatization was performed using UV spectroscopy at 252 nm wavelength. The intra-assay & intermediate precision performed with the % RSD of less than 2.0%.

INTRODUCTION: To achieve an effective therapeutic level in humans/animals, in present scenario novel drug delivery system (NDDS) is a perfect adaptable tool compare to the normal conventional supply system. NDDS attained different versions like: controlled drug distribution method, targeted drug distribution system, moderated drug distribution arrangement, magnetic drug distribution line, thin film delivery system, micro-emulsion dosage delivery system, acoustic targeting dosage delivery system, drug delivery to the brain, bovine submaxillary mucin coatings system, retrometabolic medication distribution root and unbalanced membrane capsule *etc.*

The targeted drug distribution is a phenomenon followed by drug delivery specialist to carry the active entity to the body part where exactly the ailment occurred using active and passive targeting ¹. Controlled drug distribution process delivers the medicine at a fixed ratio in stipulated extended time when it administered through locally and systematically, which results in the drug act in reproducible phenomenon throughout the course ².

In recent scenario, modulated drug distribution process is an emerging approach to delivery medicine using engineered smart micro carriers, which seems to surpass a priori of monitoring regards current methods of delivering medicines. Magnetic drug distribution system by particulate transporters are a very effective and efficient approach to deliver the drug at the final destination of our body part. The advantage of this method is high dose delivery possible towards the chemotherapy/radiological treatment for tumor treatment without creating some side effects to the

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| <p>QUICK RESPONSE CODE</p>  | <p>DOI: 10.13040/IJPSR.0975-8232.10(12).5650-56</p> <hr/> <p>This article can be accessed online on www.ijpsr.com</p> <hr/> <p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(12).5650-56</p> |
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normal tissues³. The most spreadable mental disorder in recent days for human population is depression & it is defined as a collection of different types of illness, and mental inability, the brutality of this disorder depends from mild to severe as it is time-dependent case⁴.

This disorder ranked globally in the top as the largest contributor in the non-fatal health issues. According to Global Health Estimates, approximately 7.5% of global population is being suffered from this disability, close to 2% of people are getting possible chance to recover from this mental disorder⁵ the graphical representation presented in the **Fig. 1**. In addition to that, it is predictable to be the second foremost reason for all diseases⁶. Internationally, the percentage of the population with depression is assessed to be 4.4% out of which 5.1% and 3.6% will be the females and males respectively. According to the WHO constituencies, the proportion varies from as low as 2.6% among males in the Western Pacific Area to 5.9% among females in the African Region.

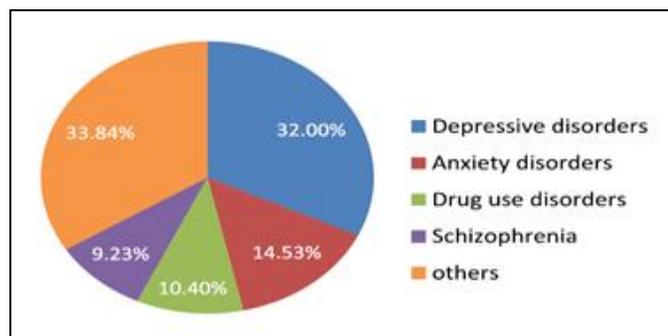


FIG. 1: DALYs DUE TO MENTAL AND SUBSTANCES ABUSE

The Sustained distributed dosage form termed differently similar to sustained action, prolonged the action, controlled release, extended action to pin the standards on its mechanism. Though it named differently, the core aim is to design a formulation to achieve prolong therapeutic effect by continuously releasing medication through a long period after administration of a single dose by minimizing the side effects.

Bupropion Hydrochloride (BPH) is an antidepressant medication chemically this entity known as 2-(tert-butylamino)-1-(3-chlorophenyl) propan-1-one hydrochloride **Fig. 2**¹⁰, BPH is a colorless crystalline powder, highly polar and soluble in water, it is used to treat major depressive

disorders and seasonal affective disorders. Additionally, it helps people to stop smoking. BPH can improve the effects of the natural neurotransmitter in the human brain.

The detail literature review expressed that, BPH & its metabolites estimation was done in human plasma by using LC method¹⁰, review of Bupropion for Smoking Cessation¹³, bupropion for main depressive disorder: Pharmacokinetics & formulation study¹⁴, pharmacokinetics of bph in haemodialysis patient who smokes¹⁵, Tandem mass spectroscopy diagnosis of Bupropion & its metabolites in human, mouse, rat plasma using a monolithic column¹⁶, stereoselective analysis of BPH & Hydroxybupropion in human plasma & urine by LC-MS-MS¹⁷, simultaneous quantitative determination of BPH and its three major metabolites in human umbilical cord¹⁸, analysis of retention behavior of BPH in reverse phase ion pair liquid chromatography¹⁹, analytical strategies for the fortitude of norepinephrine reuptake inhibitors in pharmaceutical formulations²⁰, High Pressure Liquid Chromatography (HPLC) assay for bupropion and its major metabolites in human plasma²¹, titrimetric and spectrophotometric assay of BPH in pharmaceuticals using mercury II nitrate²², pharmacokinetic optimization of sustained-release bupropion for smoking cessation²³, pharmacokinetics of bupropion and its major basic metabolites in normal subjects after a single dose²⁴, pharmacological significance of the species differences in BPH metabolism²⁵.

RP-HPLC method development and validation for the determination of BPH in a solid dosage form¹¹, Stress degradation studies of BPH & development of a UV spectroscopic method in bulk & pharmaceutical dosage form¹² through the UV analysis work related to present study the derivative spectroscopy approach was not attempted to confirm the intrusive elements from the excipients of solid dosage form, in addition to that the stability parameters were not been screened to assure the analysis time with minimum usage of reference standard and sample solution, In the other reported methods BPH and its metabolites were estimated using higher end equipment which produce high accurate result with low concentration correspondingly the cost of analysis also high.

The present work aims to develop simple, sensitive stability indicating the method to develop a model to estimate BPH in the solid dosage form by using derivative spectroscopy tool to avoid the excipients interferences in the established assay method.

MATERIALS AND METHODS: The BPH reference standard obtained from Clearsynth, Mumbai, as gift sample. Spectroscopy grade Methanol from Sigma-Aldrich Chemicals Company, Millipore water from Milli-Q® Integral Water Distillation System. Shimadzu-1700 double beam UV-Visible spectrophotometer with 0.5 nm band width with UV probe 2.31 software. BPH tablet dosage form procured from the local commercial market.

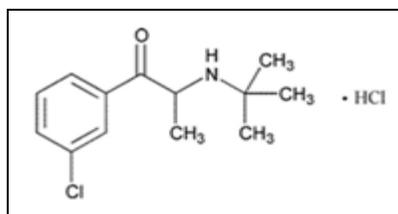


FIG. 2: THE CHEMICAL STRUCTURE OF BUPROPION HYDROCHLORIDE

Preparation of Assay Solution: Weigh 20 tablets separately and then take the average weight of the total tablet. Crushed all the tablet using Mortar & Pestle. From their weighing 10 mg of subtle crushed tablet powder and transfer into a 10 ml volumetric flask and add a little amount of Millipore water for dissolving purpose. Sonicate this for 10 minutes and volume makes up with Millipore water. Then filter the solution using Whatman filter paper and separate the filtrate into another volumetric flask. With concentration 1 mg/ml.

Preparation of Working Standard Solution: From the reference standard solution pipette out 1 ml and transfer to another 10 ml volumetric flask and made up the volume with Millipore Water. And the concentration of the solution is 100 µg/ml.

Preparation of Standard Stock Solution: To perform the assay of the particular dosage product, made 10 dilutions with different concentration from the standard working solution (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 µg/ml).

Method Validation: As described the above suitable condition, the calibration lines were

obtained using standard solutions of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/mL. Bupron® SR. Linear regression analysis was used to calculate the slope, intercept, and coefficient of determination (r^2) of each calibration line.

The accuracy and precision of the methods were determined as documented by ICH Q2(R1) guideline. Multiple analysis of BUP at ten different known concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/mL) were analyzed daily (intra-day) and repeated for three days (inter-day) using the validated analytical procedure. The outcomes were calculated as mean \pm standard deviation & % RSD for both intra-day and inter-day analysis.

Assay: Assay procedure is to characterize the quality of bulk pharmaceuticals. In the field of drug analysis, bulk drug materials analysis, intermediate occurs with their synthesis, impurities, degradation products, and metabolites of biological samples is a very important area of research. Assay procedures are mainly used to measure the analyte present in a given sample. This study aims to obtain data that can contribute to high quality, maximal efficacy, and safety of drug therapy⁷.

Typical validation characteristics which should be considered are listed below:

- Accuracy
- Precision
 - ✓ Intermediate Precision
 - ✓ Interday Precision
- Linearity
- Range
- Recovery Study
- Stability study

Precision: The precision is the analytical procedure which expresses the closeness of agreement between sequences of measurement acquired from multiple sampling of the same sample under the given conditions⁸.

Linearity: Linearity study was done for testing the drug absorbance phenomenon in accordance with various concentration range. Also to merge the stability of the drug.

Range: Range is defined as an analytical procedure which shows the suitable level of precision, accuracy & linearity in a proper interval of upper and lower concentration analyte limit.

Recovery Study: Accuracy was being assessed using a minimum of 9 determinations over a multiple concentration range. Accuracy should be determined the assay of previously added of analyte in the sample or difference between the mean and the accepted true value.

Derivative Spectroscopy: Chemical derivatization is the part of secondary spectrophotometric analysis procedure to determine the qualitative and quantitative measurement of a particular in the sample solution. Derivative spectroscopy is the changes in the higher range of absorption as regards to wavelength for multiple analysis. If a spectrum is expressed as absorbance, A , as a function of wavelength, $f(\lambda)$, and the derivative spectra are:

Zero Order:

$$A = f(\lambda)$$

First Order:

$$dA/d\lambda = f'(\lambda)$$

Second Order:

$$d^2A/d\lambda^2 = f''(\lambda)$$

A first-order derivative is the rate of change of absorbance concerning wavelength as the spectra start and end at zero. So, we can say it passes through zero at the same wavelength as λ_{\max} of the absorbance band. At the same wavelength of inflection points of absorbance band, the positive and negative bands occur. The distinctive feature of a second-order derivative is a negative band with a minimum at the same wavelength, but it is being shown maximum if we compare with the zero order band. Sometimes it also shows positive bands on either side of the main band, but it depends on performed sample solution⁹.

Obtaining of Derivative Spectra: Derivative spectra can be obtained by optical, electronic, or mathematical methods. Optical and electronic techniques were used on early UV-Visible

spectrophotometers but have largely been superseded by mathematical techniques. The advantages of the mathematical techniques are that derivative spectra may be easily calculated and recalculated with different parameters, and smoothing techniques may be used to improve the Signal-to-noise ratio.

RESULTS AND DISCUSSION: UV spectroscopy was applied for the concurrent estimation of marketed formulation of Bupropion hydrochloride. Since, it is the authoritative analytical technique to perform the pharmacokinetic study stability, selectivity & accuracy of a test solution. The essential to have this derivatization study is to the improvement of a varied collection of reaction and procedures. Thus the main fundamentals behind derivatization reactions are complete the reactive molecule to readily determinable compounds with keeping the properties and concentration same as original compounds.

Determination of Maximum λ_{\max} through UV-Spectroscopic Method: The very beginning step is to know & perform the maximum wavelength of that particular dosage form to perform the assay so that we can get the best absorbance value.

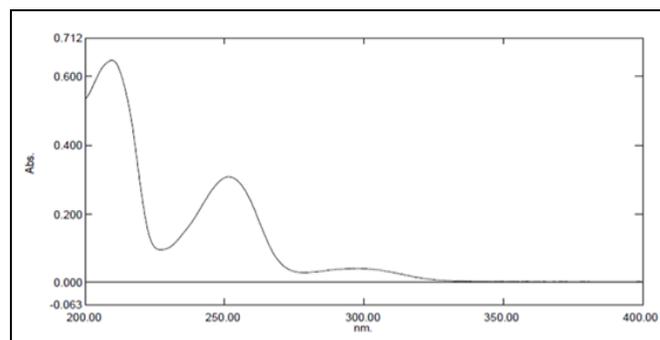


FIG. 3: MAXIMUM WAVELENGTH OF PREPARED SOLUTION

Above spectra indicate the maximum wavelength of prepared solution which was run through a UV spectra method & we got the maximum wavelength add the UV spectra shown in **Fig. 3**.

Precision: Precision is the method to accumulate the closeness of individual series of solution to various aliquots of concentration of the solution.

Intra-Day Precision: Intra-day precision was performed with multiple concentration range of 5,

10, 15, 20, 25, 30, 35, 40, 45 & 50 µg/ml. After performing the intraday concentration, the result of % RSD is less than 2 %, which will consider being low variance or within the proper limit.

TABLE 1: PRECISION STUDY

| S. no. | Conc. (µg/ml) |
|--------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 |
| 1 | 0.083 | 0.159 | 0.237 | 0.3 | 0.377 | 0.485 | 0.62 | 0.685 | 0.707 | 0.847 |
| 2 | 0.086 | 0.156 | 0.24 | 0.301 | 0.382 | 0.482 | 0.621 | 0.68 | 0.702 | 0.843 |
| 3 | 0.084 | 0.162 | 0.235 | 0.297 | 0.382 | 0.487 | 0.624 | 0.684 | 0.706 | 0.844 |
| STD V | 0.0015 | 0.003 | 0.0025 | 0.0020 | 0.0029 | 0.0025 | 0.0020 | 0.0026 | 0.0026 | 0.0021 |
| Mean | 0.0843 | 0.162 | 0.2373 | 0.2993 | 0.3803 | 0.4847 | 0.6217 | 0.683 | 0.705 | 0.8447 |
| % RSD | 1.81 | 1.85 | 1.06 | 0.69 | 0.76 | 0.52 | 0.33 | 0.39 | 0.38 | 0.25 |

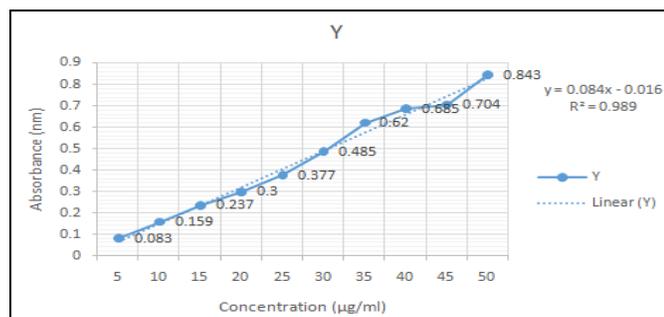
Inter-day Precision: Inter-day precision was determined by the analysis of one aliquot sample on three consecutive days. The % RSD result is under 1%, which indicates a low variance of the method.

TABLE 2: INTER AND INTRA DAY STUDY

| | 25 µg/ml | | |
|-------|----------|--------|--------|
| | Day 1 | Day 2 | Day 3 |
| 1 | 0.491 | 0.489 | 0.49 |
| 2 | 0.493 | 0.486 | 0.488 |
| 3 | 0.491 | 0.488 | 0.489 |
| STDV | 0.0012 | 0.0015 | 0.001 |
| Mean | 0.4917 | 0.4877 | 0.489 |
| % RSD | 0.0012 | 0.0015 | 0.2045 |

TABLE 3: NORMAL LINEARITY RESPONSE

| S. no. | Concentration (µg/ml) | Absorbance at 252 nm |
|--------|-----------------------|----------------------|
| 1 | 5 | 0.083 |
| 2 | 10 | 0.159 |
| 3 | 15 | 0.237 |
| 4 | 20 | 0.3 |
| 5 | 25 | 0.377 |
| 6 | 30 | 0.485 |
| 7 | 35 | 0.62 |
| 8 | 40 | 0.685 |
| 9 | 45 | 0.704 |
| 10 | 50 | 0.843 |

**FIG. 4: LINEARITY CURVE FOR MARKETED DOSAGE OF BPH**

Linearity: Linearity was determined to obtain the linear relationship between the detector response and the concentration of the analyte required. From

the linearity curve, got the regression value of 0.99, which represents the goodness of fit. The normal linear response is shown in Fig. 4.

Percentage (%) Purity: Performance the percentage purity to determine the pure compounds in an impure sample.

Sample solution absorbance = 0.380

Standard solution absorbance = 0.377

Standard concentration = 25 µg/ml

Test concentration = 25 µg/ml

% Recovery = (Sample absorbance) / (Standard absorbance) × (Standard concentration) / (Test concentration) × 100

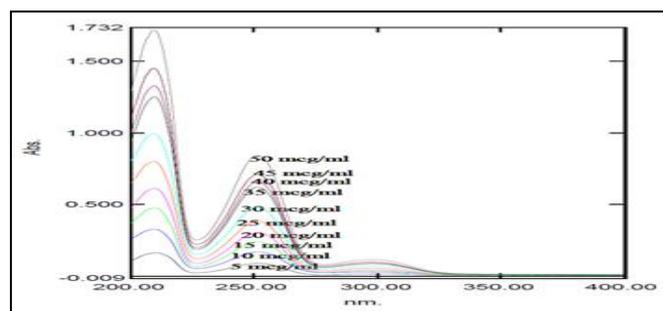
$$= 0.380 / 0.377 \times 25 / 25 \times 100$$

$$= 100.788\% \text{ w/w}$$

Derivatization Study:

Normal Spectrum Derivatization Analysis:

Normal spectrum derivatization, also known as zero-order spectra. The zero derivative spectra shown in Fig. 5. The different response curve occurs with different types of concentration of aliquots of the sample.

**FIG. 5: NORMAL SPECTRA OF BPH**

First Derivative Spectrum Analysis: A first-order derivative is the rate of change of absorbance concerning wavelength. The curves are finished at zero.

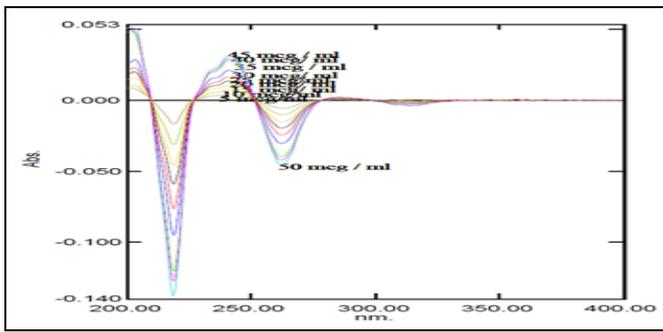


FIG. 6: FIRST DERIVATIVE CURVE OF BPH

Linear Response for First Derivatization Spectra: These are the following absorbance with the response of concentration we got after the first order kinetic derivatization study. The regression value for the first order kinetic derivative study was observed 0.99, which indicates the good of a fit.

TABLE 4: LINEAR RESPONSE FOR FIRST DERIVATIZATION

| S. no. | Concentration (µg/ml) | Absorbance (nm) |
|--------|-----------------------|-----------------|
| 1 | 5 | 0.006 |
| 2 | 10 | 0.01 |
| 3 | 15 | 0.016 |
| 4 | 20 | 0.02 |
| 5 | 25 | 0.024 |
| 6 | 30 | 0.03 |
| 7 | 35 | 0.04 |
| 8 | 40 | 0.044 |
| 9 | 45 | 0.049 |
| 10 | 50 | 0.051 |

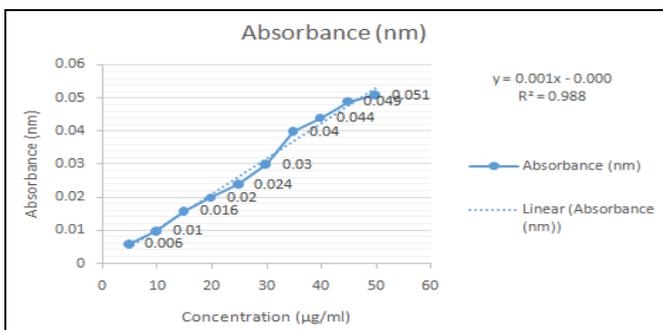


FIG. 7: LINEARITY CURVE OF FIRST DERIVATIZATION SPECTRUM ANALYSIS

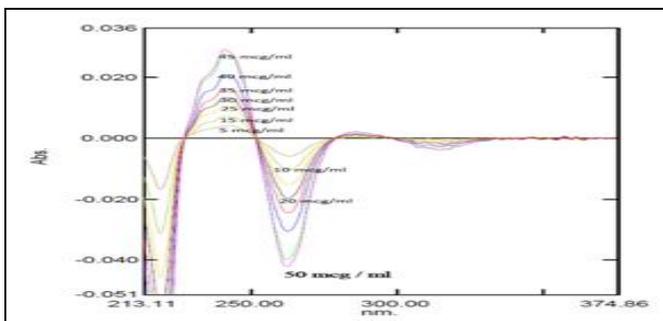


FIG. 8: LINEARITY CURVE OF SECOND DERIVATIVE SPECTRUM ANALYSIS

Second Derivative Spectrum Analysis: In the second order derivative study, we got the negative spectra at the same wavelength.

Linear Response for second Derivatization Spectra: These are the following absorbance with the response of concentration we got after the second order kinetic derivatization study. The regression value for the first order kinetic derivative study was observed 0.98, which indicates the good of a fit.

TABLE 5: LINEAR RESPONSE FOR SECOND DERIVATIZATION

| S. no. | Concentration (µg/ml) | Absorbance (nm) |
|--------|-----------------------|-----------------|
| 1 | 5 | -0.001 |
| 2 | 10 | -0.001 |
| 3 | 15 | -0.002 |
| 4 | 20 | -0.003 |
| 5 | 25 | -0.004 |
| 6 | 30 | -0.004 |
| 7 | 35 | -0.005 |
| 8 | 40 | -0.006 |
| 9 | 45 | -0.007 |
| 10 | 50 | -0.007 |

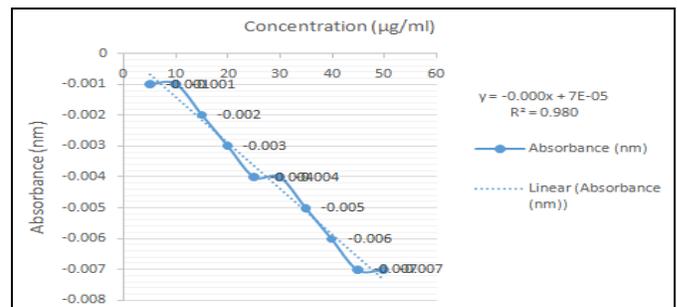


FIG. 9: REGRESSION LINE & VALUE OF SECOND DERIVATIZATION SPECTRA

CONCLUSION: An innovative approach called derivatization spectroscopy was established as simple, sensitive, stability indicating method & to estimate Bupropion hydrochloride in the solid dosage form by using derivative spectroscopy tool to avoid the excipients interferences in the developed assay method. The results obtained were found to be precise and exclusive. Confirm the interfering substances from the excipients of solid dosage form, in addition to that the stability parameters to assure the analysis time with the minimum usage of the reference standard and sample solution.

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CONFLICTS OF INTEREST: Nil**REFERENCES:**

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